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MORPHOLOGICAL AND ISOENZYME VARIATION IN *RHODODENDRON OCCIDENTALE* (WESTERN AZALEA) (SECTION *PENTANTHERA*; ERICACEAE)

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ABSTRACT

Morphological and isoenzyme variation among populations of western azalea, *Rhododendron occidentale* (Torr. & A. Gray) A. Gray, were examined. Three regional parapatric groups were revealed: 1) the northern California outer North Coast Ranges; 2) the northern California and southern Oregon Klamath Ranges; and 3) the central California Sierra Nevada and southern California Peninsular Ranges. A highly variable but generally intermediate fourth group is restricted to ultrabasic substrates (serpentine) in the middle and inner North Coast Ranges of California. It is comprised of populations with recombined morphologies and alleles that were otherwise restricted to one or more of the three groups above. A revised intraspecific treatment is proposed, with the three regional groups above recognized as varieties. These are: *R. occidentale* (Torr. & A. Gray) A. Gray var. *occidentale* (outer North Coast Ranges), *R. o.* var. *paludosum* Jeps. (Klamath Ranges), and **Rhododendron. occidentale** var. **californicum** (Torr. & A. Gray) Hrusa comb. et stat. nov. (Sierra Nevada and Peninsular Ranges). Lectotypifications of *Azalea occidentalis* Torr. & A. Gray isotypes at PH and GH, *Azalea californica* Torr. & A. Gray in Durand, and *Rhododendron sonomense* Greene (NDG) are also provided.

Key Words: Azalea, isozyme, lectotype, morphometric, population, *Rhododendron occidentale*.

In addition to its large and fragrant flowers *Rhododendron occidentale* (Torr. & A. Gray) A. Gray has long been recognized for its diversity of growth habits, floral pigmentations, and vegetative forms (Kellogg 1855; Wilson and Rehder 1921; Jepson 1939, Munz and Keck 1959; Mossman and Smith 1969; Kron 1993). The only more or less invariant characteristic is the presence of a yellow nectar guide on the upper corolla limb. It has been suggested that this variation indicates the existence of multiple species (Mossman and Smith 1969; Mossman 1977). Other observers have interpreted these polymorphisms as randomly distributed genotypes or as environmentally induced (Breakey 1960). The most recent systematic treatment of section *Pentanthera* (Kron 1993) did not examine the presence or patterns of intraspecific variation in *R. occidentale*, and the same can be said of the discussion in Wilson and Rehder (1921). Indeed, there has been no detailed accounting of its regional diversity.

In horticultural circles its visible morphological variation has made *R. occidentale* a favorite of azalea breeders. Middle nineteenth- to early twentieth-century selections were prominent in these breeding programs (Mossman 1974). The results can be found for sale as the Knapp Hill and Exbury hybrid series. More recently, enthusiasts have been seeking out novel wild forms. Especially popular are those with unusual corolla shapes and color variations (Mossman 1974, 1977), which are sold with names such as 'Humboldt Picotee', 'Tatum's Pink', or 'Double Dig Twelve' (Jones et al. 2007). Accompanying the reports of these variants' discoveries were poorly documented assertions regarding polyploidy; wild distributions; regional variation patterns; and edaphic, moisture, and temperature tolerances in wild populations (Breakey 1960; Mossman and Smith 1968; Mossman 1972, 1974, 1977). Ultimately, experimental evidence for any of these interpretations is lacking.

Geographic Distribution

Rhododendron occidentale is distributed within the Coast Ranges of California, the Klamath Ranges of northern California and southern Oregon, the Sierra Nevada, and the Peninsular Ranges of southern California. Its distribution is largely coincident to the California Floristic Province (CFP) (Raven & Axelrod 1978) with its northernmost populations only about 75 miles north of the CFP along the Umpqua River in Douglas Co., Oregon. It is found at elevations extending from sea level to near 2800 m. Throughout its range it is restricted to sites of permanent moisture, although these may be subsurface sources. It is frequently found growing on ultrabasic soils, predominantly serpentine, particularly in the Klamath ranges and inner North Coast Ranges. Except for a single location at the northern tip of the Gabilan Range in

Monterey Co., *R. occidentale* is absent from the California South Coast Ranges and Transverse Ranges. It is presumed that the warming and drying of the post-Pleistocene eliminated it from these relatively dry mountains. Claims that *R. occidentale* has native populations in the Puget Sound area, the Olympic Peninsula, and on Mount Rainier in Washington (Mossman 1974) require confirmation.

This paper will present quantitative and qualitative analyses that describe the patterns of morphological and isoenzyme variation among wild populations of *R. occidentale*. A taxonomic treatment that accounts for the species' natural variation patterns will also be proposed.

Materials and Methods

Sampling

Populations representing the species' full geographic range were analyzed. Thirty-six populations were used for the morphological analysis, and 37 populations were used for the isoenzyme analysis (Fig. 1 and Appendix 1).

All but three population samples contained a minimum of 25 individuals. The three smaller samples came from critically situated populations that did not contain that many individuals. *Rhododendron occidentale* is not rhizomatous, nor does it sprout from the roots. It may, however, form clones when fallen trees or branches press plants to the ground and adventitious roots develop. There was adequate visible variation among individuals in both growing and dormant structures so that duplicate clone collections were readily avoided. Sampling was random throughout except in the case of the smaller populations where every individual was examined.

The morphological samples included both spring-collected flowering material and winter-collected dormant inflorescence buds. Thus the dormant and flowering collections used for morphological examination did not necessarily represent the same individuals. This did not affect the analyses because the characters were defined as population summaries. Specimens for isoenzyme analysis were either fresh, spring-collected corolla tissue or mature, dormant vegetative branch tips.

The sampled populations are mapped in Figure 1. Locality descriptions and sample sizes are listed in Appendix 1, and representative vouchers from those populations have been deposited at CDA and DAV. All statistical analyses were performed in JMP 5.1 (SAS Institute).

Morphometric Data

Traditional morphological classifications of *Rhododendron* have emphasized variation in floral pigmentations and vegetative trichome types and their positions, in addition to quantitative physical data (Sleumer 1949; Kron 1993). The first two proved particularly useful in this study.

Quantitative data were taken from specimens collected, pressed, and dried specifically for these analyses. Because of the variable bilateral form of *Rhododendron occidentale* corollas, special handling and pressing of individual flowers after their removal from the inflorescence was necessary for consistent measurement of corolla tube and limb relationships. For the quantitative characters, values were defined as the mean of four measurement repetitions per structure per individual. As much as possible each measured structure was standardized by plant position and developmental stage. Phenotypic plasticity within leaf size, shape, venation patterns, and trichome length were demonstrated in a preliminary unpublished study (Hrusa 1991). Seed and capsule features including seed wing shape and capsule sizes and shapes were highly variable within individuals and were not used.

The raw measurements were taken at various scales. These were ranged to between 0 and 1 using Gower's Transformation (Sneath and Sokal 1973). The population-based quantitative characters were defined as the proportion of the range for a given population that fell in two of three equal classes that represented the upper and lower one-third of the structure's among-population range. Statistically, the central of the three quantitative classes for each character is always correlated to one of the outside thirds, and was excluded.

The qualitative character states were defined as their frequency within each population sample. Corolla coloration data were acquired from fresh material. Trichome position and density data were taken from dried specimens. The quantitative and qualitative characters are listed in Table 1.

Isoenzyme Data

Soluble enzymes were extracted from fresh plant samples and electrophoresed on horizontal starch gels composed of 12% hydrolyzed potato starch and 3% sucrose. Gel and electrode buffers were composed of 0.0009 M L-histidine-0.0003 M citric acid and 0.065 M L-histidine-0.019 M citric acid, both at pH 5.7. Electrophoresis proceeded for 13 hours at 3.5 watts.

Allelic variants were classified and identified by their relative migration distance against a standard allele at each locus. This allele was one present in every population and was usually the most common. Homologies among the variant electromorphs were determined by comparing them on common gels.

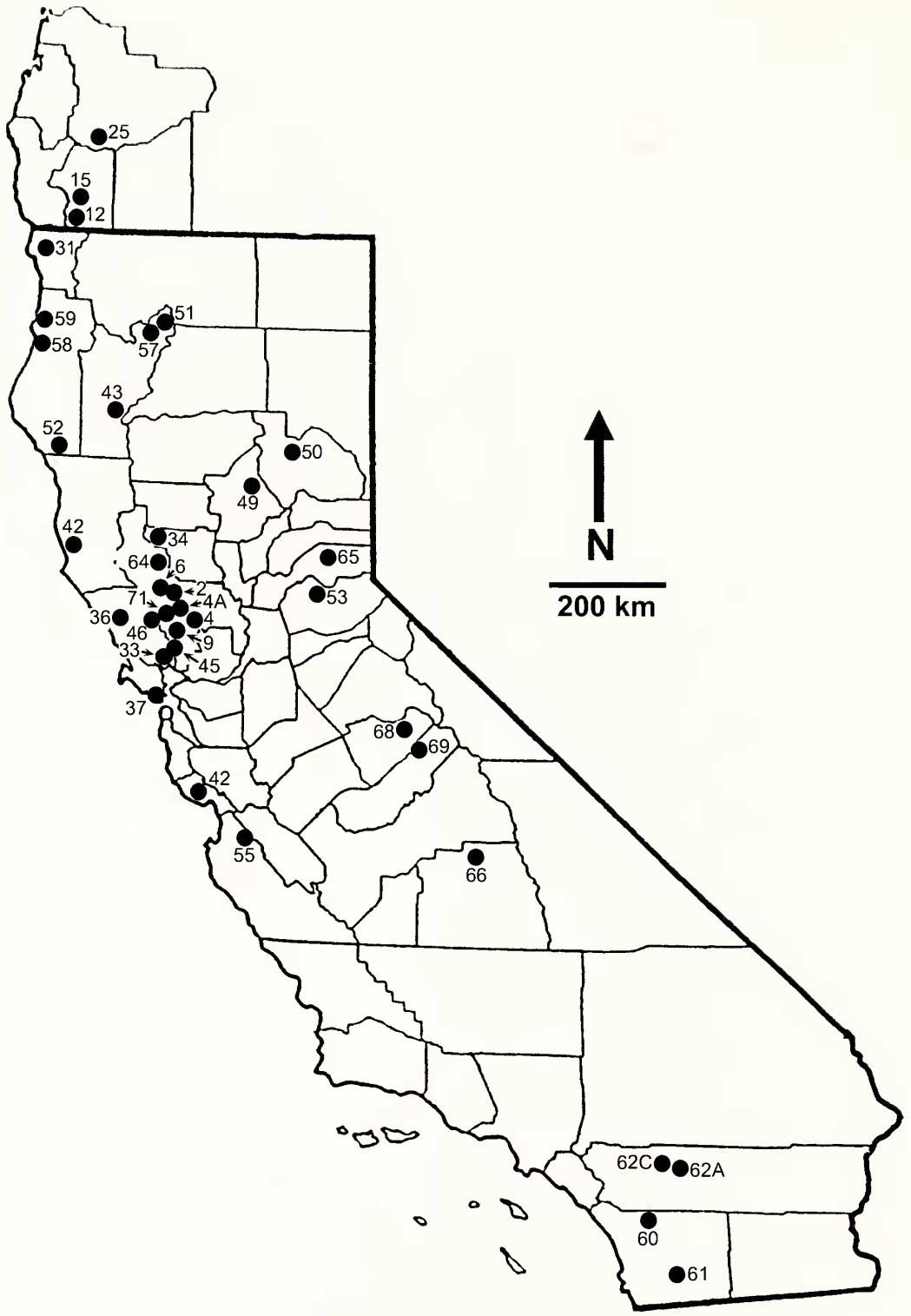

FIG. 1. Populations of *Rhododendron occidentale* used in the morphological and isoenzyme analyses. See Appendix 1 for geographic location and sampling details.

RESULTS

Morphological Variation

Thirty-eight outwardly visible features (21 quantitative and 17 qualitative) were described and measured or assessed (Table 1). The among-population comparisons were performed through principal components analysis of the among-population correlation matrices.

The first three principal components described 84.9% of the total variation, with four clusters of populations evident. These are plotted in Figure 2 and mapped in Figure 3. The Sierra Nevada and Peninsular Range populations (SNPR) grouped together. The populations of the Klamath Ranges (KR) and outer North Coast Ranges (ONCR) ordinated apart from both each other and from the SNPR populations. With two exceptions, the inner North Coast Range Serpentine (INCRS) populations plotted between the ONCR, KR, and SNPR populations, much as they are situated geographically (Fig. 3). The exceptions, (P-64, Kilpepper Creek, Lake Co.; and P-34, Stony Creek, Colusa Co.) grouped among the SNPR populations. These two populations shared with those of the SNPR the characteristics of relatively long, generally white-colored corolla tubes, and a pubescent leaf abaxium.

Stepwise discriminant analysis was used to evaluate which characters were useful in distinguishing the four groups in Figure 2. The results indicated that individuals from the KR and ONCR populations shared combined glandular and eglandular multicellular trichomes on the leaf

TABLE 1. DEFINITIONS OF MORPHOLOGICAL FEATURES USED IN THE MULTIVARIATE ANALYSES. Asterisks (*) denote subset of nine systematically useful characters. Quantitative characters were defined as described in the text. Qualitative characters were defined as their frequency within the population sample.

Quantitative	Qualitative	
1. Ovary length: classes 1, 3	11. Perianth tube: dark pink	*20. Leaf abaxium, secondary vein trichomes: absent
2. Anther length: classes 1, 3	12. Perianth veins: pink base to summit	21. Leaf margin trichomes: mixed glandular and strigose
3. Corolla length: classes 1, 3	13. Perianth veins: white except for vein tip	*22. Floral bud-scales, margin trichomes: glomerate
4. Corolla tube length: classes 1, 3	14. Perianth limb: pink	*23. Floral bud-scales, margin trichomes: ciliate
5. Corolla length/tube length ratio: classes 1, 3	15. Perianth limb: white	*24. Young twigs, vestiture: densely pubescent
6. Inflorescence bud length: classes 1, 3	*16. Leaf abaxium, midvein trichomes: mixed glandular and strigose	*25. Young twigs, vestiture: glabrous
7. Calyx lobe length, longest: class 1	17. Leaf abaxium, midvein trichomes: glandular	*26. Leaf abaxium, surface: single-celled pubescence
8. Calyx lobe length, shortest: class 1	18. Leaf abaxium, secondary vein trichomes: mixed glandular and strigose	27. Floral bud bract, surface vestiture: pubescent
9. Calyx lobes ratio, length of longest/shortest: class 1	*19. Leaf abaxium, secondary vein trichomes: strigose	28. Floral bud bract, surface vestiture: glabrous
*10. Leaf abaxium, multicellular trichomes per 25 mm^2: class 1 (<7) and 3 (>26)		

abaxial midvein and a lamina abaxium without unicellular hairs. There may occasionally be unicellular trichomes on and adjacent the midvein, but not on the abaxial surface. The ONCR populations alone were distinguished by: 1) a dense pubescence on the young twigs and dormant bud bracts; 2) winter bud bract-margin trichomes always of a glomerate-glandular type; 3) relatively large dormant flower buds; and 4) more than 25 multicellular trichomes per/25mm^2 of leaf abaxial surface. The distinguishing features are marked with an asterisk in Table 1 and with additional morphological features summarized among the groups in Table 3.

Klamath Ranges populations alone were distinguished by: 1) mostly glabrous young twigs and either glabrous or thinly ciliate bud bracts; 2) usually ciliate, occasionally glomerate winter bud bract-margins; 3) generally a pink- to red-pigmented corolla tube; 4) a short corolla tube length in relation to the throat plus limb; and 5) the lamina abaxial surface, excluding the midvein, was devoid or nearly so of trichomes. Populations from the immediate coast generally were transitional to the ONCR azaleas (glomerate bud bract-margin trichomes and less densely pubescent bud bracts and twigs). Overall the KR azaleas had the smallest corollas with the shortest floral tubes and the most pink to red pigmentations in the limb and tube. Among the KR populations, only the single northernmost and most interior was morphologically unusual (P-25, Cow Creek, Douglas Co. Oregon, Fig. 2). This population had: 1) infrequently, a unicellular pubescence on the leaf abaxium (KR azaleas are generally glabrous abaxially) and 2) midvein trichomes of a single type only (these are usually mixed strigose and glandular in the KR). The presence of these two morphological states suggests plants of the SNPR populations. However, isoenzyme alleles characteristic of that group were absent.

The SNPR azaleas had: 1) one type of multicellular leaf midvein trichome, either glandular or eglandular, not those combined as in the KR and ONCR; 2) a unicellular pubescence throughout the lamina abaxial surface; 3) secondary vein multicellular trichomes of the same type as the midvein (glandular or eglandular); 4) absent or widely scattered multicellular trichomes on the tertiary veins (when present these were $\ll$ 25/25 mm^2); 5) a non-pigmented to rarely, slightly pigmented corolla tube; 6) corolla veins rarely with more than a hint of pigmentation at the distal tip. In general, SNPR corollas were either pure white or (infrequently) had a hint of pink in the tube or vein summit; and 7) the corolla tube was comparatively long in relation to the combined limb and throat. The SNPR plants were thus particularly distinctive morphologically. Only P-50 (Butterfly Valley, Plumas Co.) of the northern Sierra Nevada had glabrous leaf abaxia and often glabrous young twigs, both features unusual among the Sierran azaleas. Population 50 plotted between the SNPR and KR populations, the same as it is situated geographically (Fig. 3).

In general, the southern California Peninsular Range azaleas had the largest and least pigmented

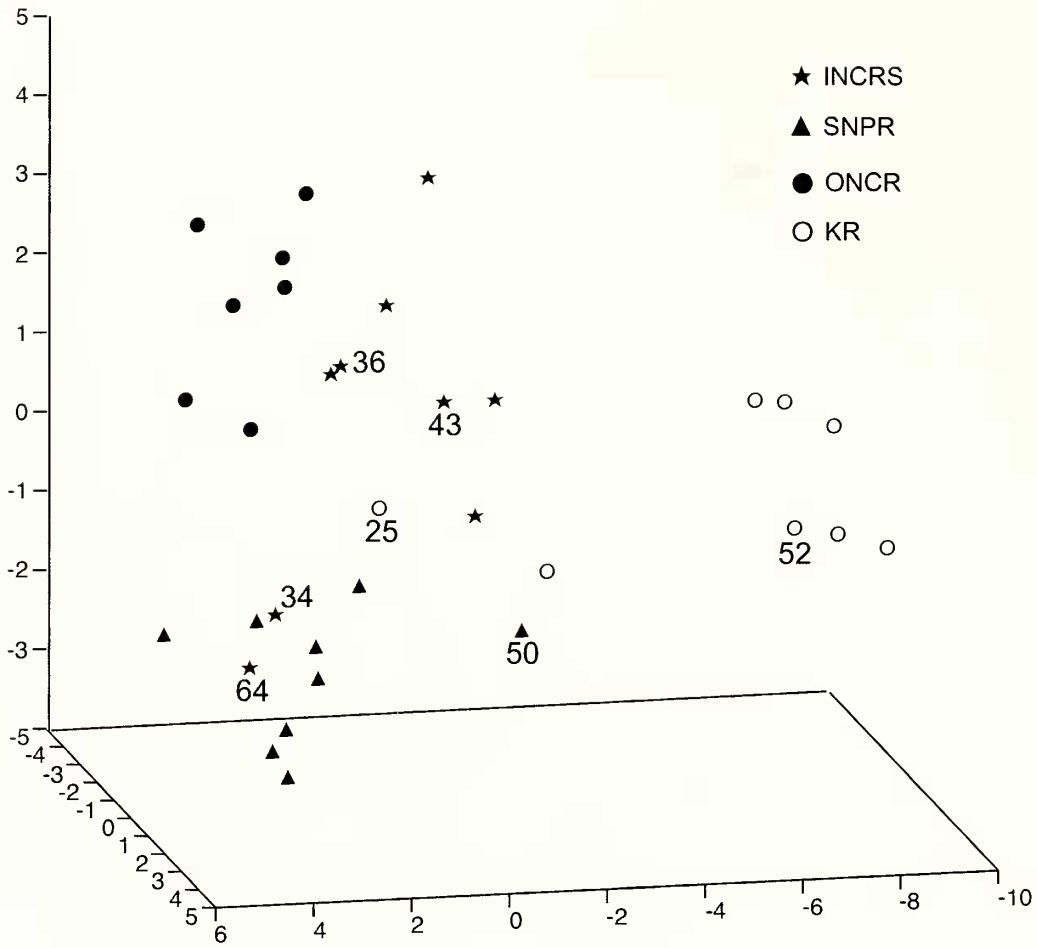

FIG. 2. Scatter plot of the first three principal component scores for the morphological dataset. Populations 64 and 34 occur in the inner North Coast Ranges on serpentine, plotted here with Sierra Nevada populations. Labeled populations are discussed in the text.

flowers with the longest floral tubes. Overall, excluding the INCRS azaleas, a cline exists from north to south in which corolla coloration diminishes and the corollas become larger overall and have relatively longer tubes.

Even excluding P-34 (Stony Creek, Colusa Co.) and P-64 (Kilpepper Creek, Lake Co.) that ordinated among the SNPR, the INCRS populations were weakly differentiated. They were distinguishable morphologically only by a shortest calyx lobe that was generally less than 1 mm in length, and sometimes nearly obsolete. Their multivariate scattering across the space between the KR, ONCR, and SNPR groups (Fig. 2) was the result of both quantitative intermediacy and mosaic patterns among the qualitative features. This variation was spread among the first three principal components and is suggestive of segregation and recombination among the analyzed features.

Definitions of three readily visible and at least partially regionally distinctive features proved problematic and were not included as population-level characters in the multivariate analyses. Darkly anthocyanous new growth was a distinctive feature of both sun- and shade-grown INCRS azaleas. Elsewhere, azaleas growing on ultrabasic-derived soils often had at least some anthocyanin pigmentation in the young twigs, particularly in plants growing in full sun. This pigmentation (and lack of same from non-pigmented populations) was maintained in greenhouse-grown seedlings, indicating it is under genetic control, at least in the INCRS (Hrusa 1991). Although the non-INCRS anthocyanin pigments were not as dark as that among individuals within the INCRS populations, this distinction could not be consistently delimited into a set of qualitative categories. Such coloration is a feature of many ultrabasic-adapted taxa (Kruckeberg 1984).

Strongly bifacial leaves that were lighter on the abaxial surface characterized many populations, mostly those of the SNPR. Several INCRS populations were similarly bifacial. Some of the latter were dimorphic for that characteristic, and in those populations the intensity of the bifacial condition was particularly variable, often varying in intensity even within individuals.

The timing of flower bud opening relative to leaf break is generally species specific within *Rhododendron* section *Pentanthera* (Kron 1993). Inflorescences may open concurrently to or after foliage maturation. In *R. occidentale* both conditions occur. Plants of the KR and most of the INCRS open flowers and leaf buds concurrently. Exceptions may occur in densely shaded plants where bud (and often leaf) break may be delayed. However, most followed the pattern of inflorescence break in concert with foliar emergence. One population from the INCRS (P-64, Kilpepper Creek, Lake Co.) broke flower buds only after new growth had fully matured; yet plants in the closely related and nearby P-34 (Little Stony

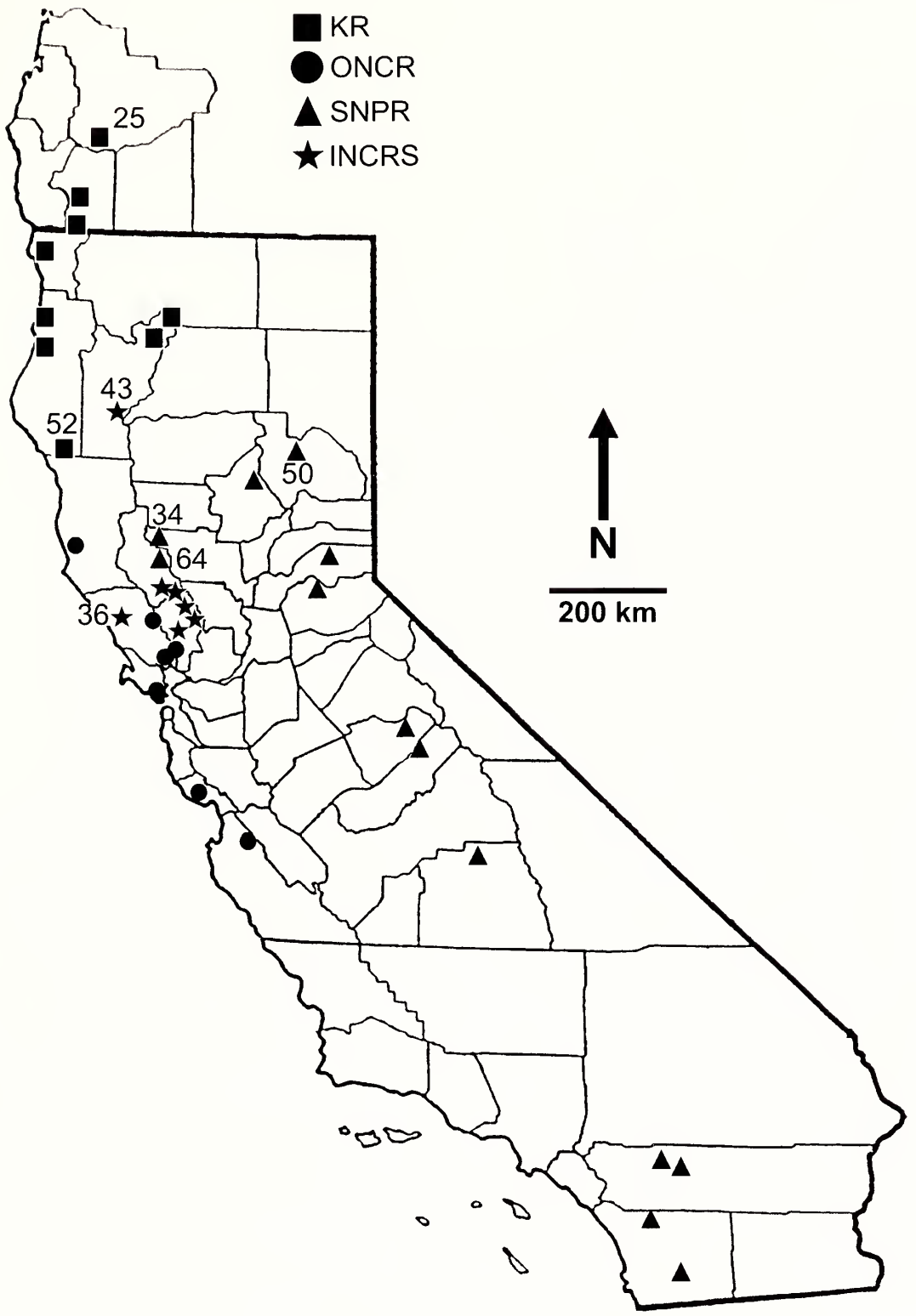

FIG. 3. Map of the population clusters based on morphological variation as identified in Fig. 2. Labeled populations are discussed in the text.

Creek, Colusa Co.) opened concurrent to bud break. With one exception each, plants of the ONCR and SNPR opened their inflorescences after the foliage had fully expanded and matured. Only the ONCR P-45 (Hogback Mountain, Sonoma Co.), growing on a highly insolated and relatively dry hillside, pushed its flower buds late-concurrent to leaf break. Again with one exception, Sierra Nevada populations flowered after leaf maturity. Population 49 (Pulga Rd., Butte Co.), from an open, south-facing serpentine slope, opened its flowers with new growth expansion. Thus, while bud and leaf break patterns are mostly consistent regionally, the exceptions suggest that local environmental conditions or selection in open habitats influence timing of flower bud break.

Isoenzyme Variation

Forty-two alleles were resolved at seven loci from four enzyme systems. The resolved variants were in malate dehydrogenase (MDH-1, MDH-2, MDH-3, 11 total alleles), phosphoglucomutase (PGM-1, PGM-2, 6 total alleles), 6-phosphogluconate dehydrogenase (6-PDH, 1 locus, 5 total alleles), and glucose-6-phosphate-isomerase (GPI-2, 20 total alleles).

At each locus there was a most-frequent (primary) allele with the remaining allele(s) at lower frequencies. The presence or absence and frequency of the alternate alleles varied widely among populations. Only in P-64 (Kilpepper Creek, Lake Co.) and P-61 (Cuyamaca Peak, San Diego Co.) did the alternate alleles at PGM

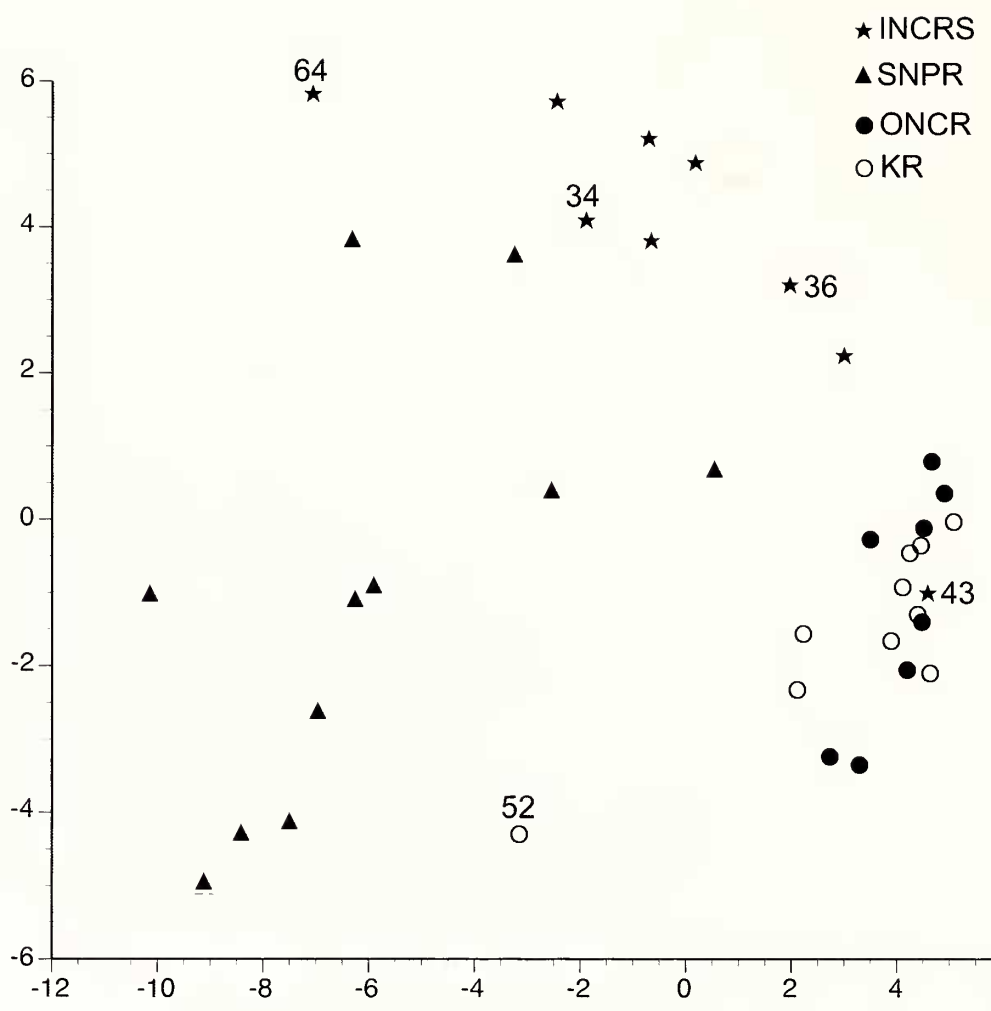

FIG. 4. Scatter plot of the first two principal component scores for the isoenzyme dataset. Labeled populations are discussed in the text.

exceed slightly the frequency of the primary allele. In no case did an alternate allele replace the primary one.

The first three principal components recovered 95.0% of the variation. Three population groups were evident, as opposed to four for the morphological data. The groups are plotted in Figure 4 and mapped in Figure 5.

The multivariate patterns were similar to those using morphology, except for the superposition of the KR and ONCR populations. With five endemic alleles the KR group might have been expected to occupy its own isoenzyme multivariate space as it did its own morphological space. That it does not is due to the presence of the nonprivate endemic alleles in only a few populations, and at relatively low frequencies.

The SNPR and INCRS populations plotted over a broader multivariate space than did the adjacent KR + ONCR cluster, due to their greater allelic diversity and higher alternate allele frequencies. Moreover, those frequencies varied considerably among the populations. In terms of allele presence or absence the SNPR and INCRS were similar. However, the shared alleles were not at similar frequencies, and this is responsible for their plotting in a slightly different, but adjacent, multivariate space. A characteristic INCRS allele was MDH-3B; although this allele was also found widely scattered at generally low frequencies among the KR, SNPR, and ONCR populations, it was in every INCRS population and at higher frequencies than all but one population outside that group.

Two populations of the North Coast Ranges also classified differently between the morphological and isoenzyme datasets. Population 43 (Wildwood, Trinity Co.), the northernmost morphologically like the INCRS group (Figs. 2, 3), contained allelic complements characteristic of the KR, not those of the INCRS.

Population 52 (Red Mountain, southern Humboldt Co.) grouped morphologically among the KR populations. However, its isoenzyme complement placed it between the SNPR and KR + ONCR populations, but on the opposite side of the multivariate space occupied by the likewise intermediate INCRS group (Fig. 4). Although P-52 contains the same SNPR and KR alleles that characterized most of the populations included here in the INCRS, it was distinguished from them by a high frequency of allele MDH-2E and a low frequency of MDH-3B, the reverse of the frequencies found in the INCRS populations. Overall P-52 has mostly KR alleles. Its SNPR connection is via relatively high frequencies of alleles PGM-2B and PGM-2A as found throughout the SNPR and INCRS populations.

In relation to the four geographic regions evident in the morphological analyses, four allele distribution patterns were discernible: 1) widespread, not ubiquitous, but occurring across geographic regions; 2) alleles common in one region, but uncommon and localized in another,

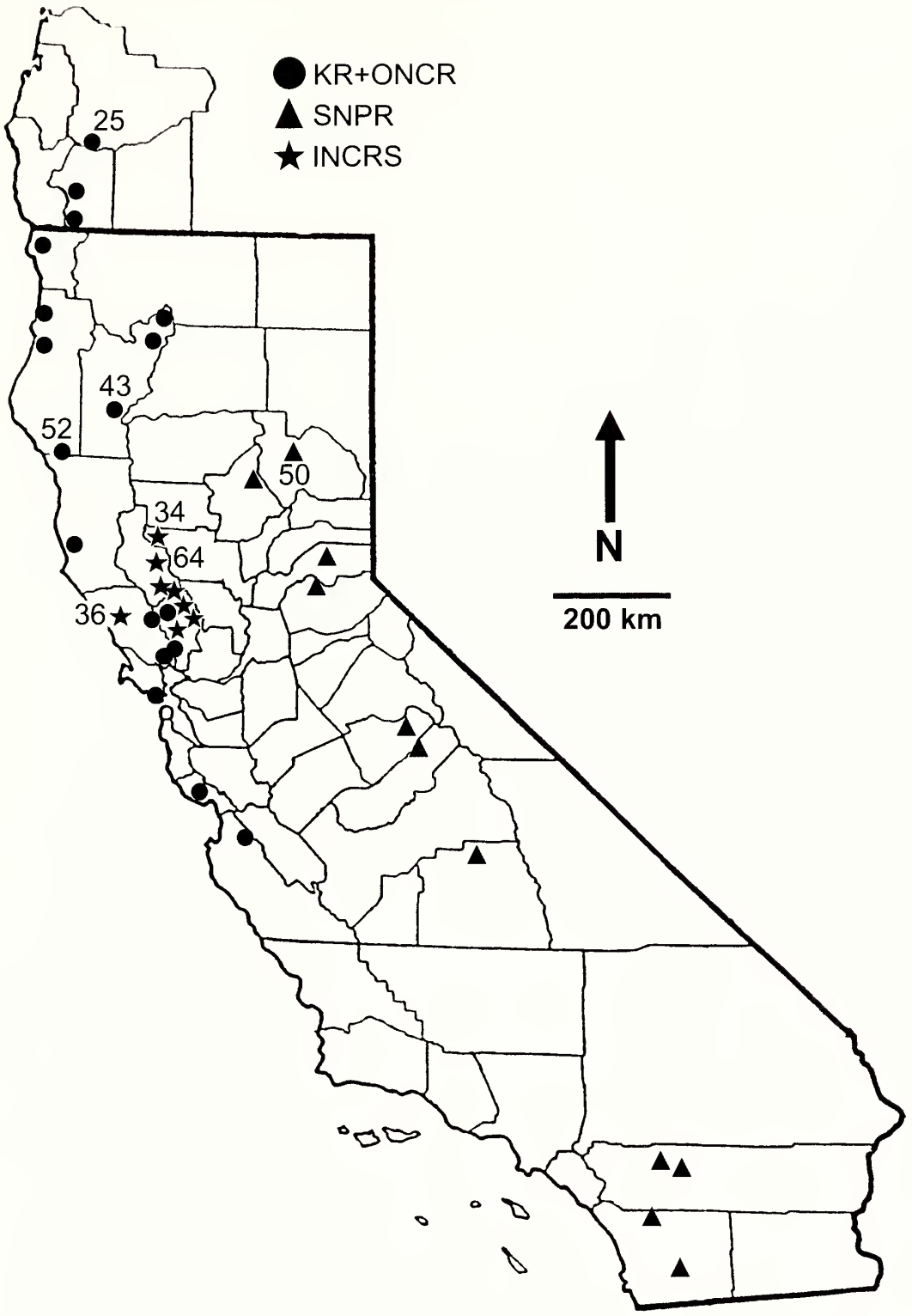

FIG. 5. Map of population clusters based on isoenzyme allele variation as identified in Fig. 4. Labeled populations are discussed or mentioned in the text.

sometimes disjunct region; 3) endemics, restricted to multiple populations within a single region, but absent elsewhere; and 4) private alleles known only from a single population.

Systematically, PGM-2 was the most distinctive. The SNPR and INCRS populations had three PGM-2 alleles (PGM-2A, PGM-2B, and PGM-2C) all at more or less equal frequencies. In contrast, the ONCR and KR populations contained exclusively or were dominated by PGM-2C, the most frequent allele at PGM-2. This PGM-2 dichotomy between the KR + ONCR and SNPR + INCRS was consistent throughout the species' range. The pattern was maintained even when the populations were in geographic proximity, a situation suggestive of restricted gene flow. Whatever is ultimately responsible for the consistently different allele frequency patterns at PGM-2 in the SNPR + INCRS versus those of the KR + ONCR, the distinction of these two combination groups is supported by allele patterns at other loci, and by morphology.

Among the 42 total alleles identified, seven were restricted to the KR region, of which two were private and five were shared among at least two populations (endemic alleles). Nine total alleles were restricted to the SNPR region; four endemics and five private. While there were also three private alleles (all P-61, Cuyamaca State Park, San Diego Co.) and one endemic allele not found in the Sierra Nevada that occurred among the Peninsular Ranges populations, there were also several alleles shared between those two regions that did not extend beyond them.

Two private alleles were in population 36 (Gilliam Creek, Sonoma Co.) of the INCRS,

TABLE 2. VARIANT ALLELES OCCURRING IN THE INCRS POPULATIONS AND THEIR DISTRIBUTION OUTSIDE THAT GROUP. 'X' = alleles found in at least one population within the group at a frequency above 0.100 (10%); '+' = alleles not found in that group at a frequency above 10%.

	Geographic regions			
Allele	INCRS	SNPR	KR	ONCR
PGI-2[a.5]	X	+		
PGI-2[b]	X	X		
PGI-2[f]	X	X		
PGI-2[g]	X		X	X
PGI-2[k]	X	X		
PGI-2[m]	X	+		X
PGM-2[a]	X	X	+	X
PGM-2[b]	X	X	+	
MDH-1[a]	X		+	
MDH-1[d]	+		X	
MDH-2[b]	X	+	+	
MDH-3[b]	X	+	+	X
6-PDH[b]	X	X		
6-PDH[d]	+	X	+	
Total shared + (%)	14	11 (78.6)	8 (57.1)	4 (28.6)
Shared at >10% + (%)	12	7 (63.6)	2 (25.0)	4 (100)
Shared, both >10% (%)	—	6 (50)	1 (8.4)	4 (33.4)

but no endemic alleles occurred among the INCRS populations. Likewise, neither private nor endemic alleles occurred within the ONCR.

DISCUSSION

From the data, four main points may be made. First, it is clear that the azaleas of the Peninsular Ranges are allied to those of the Sierra Nevada. This is not surprising given the floristic, geologic, and climatic similarities of the forested parts of the two regions (Munz 1974; Axelrod 1976; Raven and Axelrod 1978). Azalea populations of the Sierra Nevada and Peninsular Ranges shared a distinctive allele frequency pattern at PGM where the two region's populations all had relatively equal frequencies of three different alleles. In contrast only one of the two alternates was even a rare occurrence in either the KR or ONCR populations. Despite the presence of endemic GPI alleles in some Peninsular Range azaleas that suggests a long post-disjunction history, their morphological similarity to the azaleas of the Sierra Nevada, particularly the high southern Sierra Nevada, was marked. It would appear that these two regions were formerly part of a continuous interbreeding azalea population. Fossil azaleas of Pliocene age (pre-Sierra Nevada time) were found in the Chalk Hills flora of western Nevada (Axelrod 1962). These azaleas were associated with *Sequoiadendron* and other montane taxa that the western azalea is associated with in the Sierra today. However, Coast Range elements, including the Monterey Co. endemic *Abies bracteata* were also present. The leaf and capsule impressions were examined by this author, but are not assignable below section *Pentanthera*.

Second, while the KR and ONCR azaleas had similarly low frequencies of alternate alleles, the allele complements were different. Morphologically, the azaleas of these two regions were quite distinct. It may be instructive here that Kron et. al. (1993) found no allelic differences (or variants) at enzyme systems GPI and PGM between *Rhododendron canescens* (Michx.) Sweet and *R. flammeum* (Michx.) Sarg., two morphologically quite distinct species. In *R. occidentale*, some morphological intermediacy occurred along the immediate coastline north of Fort Bragg, Mendocino Co. There the pubescent new growth and outer dormant flower bud bracts of the adjacent, more southern ONCR azalea appeared as a thin pubescence in populations that otherwise were like the more frequently glabrous KR azalea. These northern coastal azaleas did not have the multicellular leaf tertiary vein trichomes that were generally characteristic of the ONCR, and their isoenzyme complements were those of the KR. As is true for the Sierra Nevada azaleas, the ONCR and KR populations are each associated with certain distinctive forest taxa such as *Sequoia sempervirens* (D. Don) Endl.and *Cupressus* (*Chamaecyparis*) *lawsoniana* A. Murray bis, respectively. Based on the fossil record (Axelrod 1962, 1976; Raven and Axelrod 1978), these taxa and their associates occupied widespread geographic regions long before being restricted to their current distribution in west coast cismontane habitats.

Third, shared endemic alleles, similar within-population allele frequencies (as at PGM-2), and certain distinctive morphological features such as the superimposition of glandular and non-glandular trichomes in KR + ONCR in contrast to restriction of those trichome types to different

TABLE 3. MORPHOLOGICAL VARIATION WITHIN *RHODODENDRON OCCIDENTALE* AMONG GEOGRAPHIC REGIONS. Parenthetical = standard deviation. *Higher number = shorter tube relative to the total corolla length.

Character	KR	ONCR	INCRS	SNPR
Corolla color, tube	white to dark pink	white to pink	gen. white, occ. pink	white/greenish
Corolla color, limb	white to dark pink	mostly white, occ. pink	white, rarely with some pink	white
*Ratio, overall corolla length/corolla tube	1.45 (0.10)	1.41 (0.06)	1.37 (0.06)	1.34 (0.05)
Multicellular trichomes, midvein	mixed glandular and eglandular, or of one type	mixed glandular and eglandular, rarely of one type	mixed glandular and eglandular, glandular, or of one type	glandular or eglandular
Multicellular trichomes, tertiary veins	absent	absent or glandular, rarely eglandular	absent or glandular	absent or glandular
Multicellular trichomes, abaxial density	zero or $\ll$ 5/25 mm^2	few to freq. >25/25 mm^2	zero, or $\ll$5/25 mm^2	zero, or $\ll$5/25 mm^2
Leaf abaxial surface, unicellular trichomes	glabrous	glabrous	pubescent or glabrous	pubescent
Inflorescence bud length (s.d.)	12.9 mm (1.81)	14.9 mm (1.84)	12.2 mm (1.59)	13.0 mm (2.56)
Inflorescence bud, vestiture	mostly glabrous	densely pubescent	variable, densely pubescent to glabrous	thinly pubescent, variable
Inflorescence bud, bract margin trichomes	ciliate	glomerate	diliate or glomerate	ciliate or glomerate
Young twig unicellular pubescence	glabrous to thinly pubescent	pubescent	glabrous to pubescent	glabrous to pubescent
Inflorescence bud break vs. veg. bud break or maturity	concurrent	post vegetative maturity	concurrent, rarely post leaf break, not post veg. maturity	post vegetative maturity, rarely semi-concurrent.

individuals in the SNPR, distinguished the azaleas of these two regions. This morphological and genetic break coincides with the floristic and paleobotanical line dividing the Klamath Ranges and the Sierra Nevada/Cascade axis (Axelrod 1962, 1976; Raven and Axelrod 1978). Again, this is evidence supporting the hypothesis that there has been a long genetic separation between the SNPR azaleas and those of the northern California coast.

Thus, although the details may be unknown, it is clear that the western azalea at present is a relict species that had a formerly more widespread and continuous distribution. Further, the possibility cannot be discounted that the morphological and isoenzyme allele groups recognizable in this study were already distinct at the time their primary associates occupied a much wider region of western North America than they do today (Axelrod 1962, 1976).

Fourth, the isoenzyme variants present among the KR and ONCR populations combine with the alleles of the SNPR azalea to form the diverse allele complements among the INCRS populations (Table 2). Moreover, a complex morphological variation more or less paralleled the isoenzyme variation. These populations are discussed in more detail below.

The "INCRS" Azalea

The INCRS azalea populations are those growing on serpentine substrata in the hot, dry, interior parts of the North Coast Ranges of Lake, Napa, and Colusa Counties. They were represented in this study by populations 2, 4, 4A, 6, 9, 34, 36, and 64 (Figs. 1, 3, 5). Population 43 shares partial morphologies with these populations but not isoenzyme alleles and is excluded from this discussion.

These azalea populations occur in permanently wet habitats on high pH ultrabasic soils within open, sunny, foothill pine–leather oak–live-oak chaparral and woodland. Such an unusual habitat for azaleas has caused them to receive some research attention (Leiser 1957; Drake 1987), and the unusual ecological situation was also a factor motivating the initiation of this study. These azalea populations on the serpentine outcrops in Napa Co. east of Mount St. Helena were misinterpreted by Jepson (1925, 1939) to be *Rhododendron occidentale* var. *sonomense* Rehder. Indeed, after his proposal of *R. sonomense*, Greene himself used that name for his own azalea collections from the same area. Although of a similar dwarfed size, he apparently did not recognize the distinctive morphological differences between the serpentine plants east of the mountain and the more coastal "Petaluma" specimen on which he had based his description. As determined in this study, the azalea populations east of Mount St. Helena contained mosaic mixtures of isoenzyme alleles otherwise found in disparate areas (Table 2). The mosaic isoenzyme allele pattern extended to the morphological variation with the complement of features in some populations like those in nearby populations; in others, the complement of features resembled the plants of distant regions. Moreover, there were distinctive mosaics of morphological characteristics and alleles within as well as among the INCRS populations. For example, by morphology, INCRS P-34 (Stony Creek, Colusa Co.) and P-64 (Kilpepper Creek, Lake Co.) plotted among the SNPR populations (Figs. 2, 3), however, by isoenzymes they plotted among the other INCRS populations (Fig. 4).

This intermediacy and among-population variability would best be explained via interbreeding among the KR, ONCR, and SNPR genotypes followed by recombination within and among the INCRS populations. The result is also a higher average number of alleles per locus: 2.11 for the INCRS populations; with 2.03 (SNPR), 1.97 (KR), and 1.48 (ONCR) for the other geographic groups. The INCRS populations intermediate geographic position parallels this genetic mixing, and lends support to the interpretation that these are the populational remnants of an ancient ecotone. The survival of azaleas in this region is apparently due to the fractured serpentine substratum that resulted in the presence of permanent springs and streams in an otherwise xeric region. The history of climatic and floristic change in this region combined with the juxtaposition of sharply delimited habitats has made it a favored area for the study of plant adaptation and evolution (Major 1967; Stebbins and Hrusa 1995).

The most distinctive population of the INCRS was P-36 (Gilliam Creek, Sonoma Co.). Morphologically this population had strongly bifacial leaves, darkly anthocyanous new growth, and corollas frequently with some pink pigmentation in the tube and limb, this latter an infrequent condition among the INCRS populations. Its isoenzyme complements included single private alleles at both PGM-2 and GPI-2, the almost equal distribution of PGM-2 alternate alleles seen in both the INCRS and SNPR populations, and GPI-2 alternate alleles characteristic of both the northern SNPR populations and of the ONCR. This latter can be explained by its proximity to ONCR populations along the coast. However, its private alleles were the only ones in the INCRS group. The private GPI-2 allele was the highest frequency GPI variant in the population, and among all the populations the PGM-2 private allele was only the fourth allele seen at that locus. Overall, this population has the aspect of a coastal form of the generally interior INCRS azalea. The distinctive morphology and isoenzyme variation of P-36 suggests some uniqueness

for the azaleas on the endemic-rich serpentine habitats of the "The Cedars" in the East Austin Creek region.

Azalea genetics and morphologies characteristic of the Sierra Nevada in the inner North Coast Ranges are not anomalous if one accepts that glycolytic isoenzyme variation parallels or contributes to physiological adaptation (Gillespie 1991). Except for the azalea populations in or near The Cedars of western Sonoma Co. discussed above, most of the INCRS azaleas are in the rain shadow of several ridges and peaks including Mount Atlas, the Palisades, Goat Mountain, and Snow Mountain. These highlands block coastal air and moisture giving their shadows a hotter and drier summer and colder winter climate than that of the outer North Coast Ranges only a few miles westward. Such isoenzyme and morphological correlation to distinctive habitats implies that the three geographic regions—KR, ONCR, and SNPR—support differently adapted genotypes. The discontinuous and mosaic patterns among INCRS alleles and morphologies are likely the end result of local population fragmentations, contractions, and re-expansions during the post-Pleistocene. Anacker et al. (2010) analyzed phylogenetic signals among plant taxa that occur in either serpentine or non-serpentine habitats. Their conclusion was that most serpentine-restricted taxa are younger than non-serpentine taxa in the same genus. The data presented here support that interpretation.

Thus, the evidence suggests a relatively recent origin for the INCRS azaleas. However, an accounting for the presence of distant Sierran alleles and two private alleles in the far western isolated serpentine Cedars region suggests that those azaleas may have a unique history within *R. occidentale*.

The described local recombinant patterns are in contrast to the variation within the more widespread ONCR, KR, and SNPR forms. These three groups are coherent in their regions, share morphologies and some alleles within (but less so among them) and would appear to have occupied the same ecologically distinct, if not allopatric regions for a long time.

Taxonomic Treatment

The three regional azalea groups determined in this study (KR, ONCR, and SNPR) were morphologically and genetically distinctive, yet vary toward each other where approaching contact, as along the northern California coast and in the northern Sierra Nevada. A treatment at varietal rank seems most appropriate as it recognizes both their distinctiveness and close relationship. As to the INCRS azaleas, further study may reveal a historical coherence worth of taxonomic recognition, but these populations are not afforded such at this time.

Rhododendron occidentale (Torr. & A. Gray) A. Gray in W. H. Brewer & S. Watson, Botany of California 1:458. 1876. *Azalea occidentalis* Torr. & A. Gray in Torr., Botany of the Expedition, Pac. Railr. Rep. 4:116. 1857.—Type: USA, California, Sonoma Co., Laguna de Santa Rosa, 1854, *J. M. Bigelow s.n.* (lectotype NY!, designated by K. A. Kron 1993; isolectotypes: GH!, PH! here designated).

The lectotype of *Azalea occidentalis* Torr. & A. Gray (Torrey 1857) designated by Kron (1993) (NY!) is clearly part of the ONCR group, as are duplicates at GH! and PH!.

Rhododendron occidentale (Torr. & A. Gray) A. Gray in W. H. Brewer & S. Watson var. *occidentale*. Synonyms - *Rhododendron sonomense* Greene, Pittonia 2:172. Sept. 1891. *Rhododendron occidentale* var. *sonomense* Rehder, Monograph of Azaleas; 127, 1921 (var. *novus* based on *Rhododendron sonomense* Greene). —Type: USA, California, Sonoma Co., "near Petaluma," May 24, 1891, *Miss Carlton s.n.*, (lectotype: here designated, left specimen, NDG 037326!, [pre-1966 Herbarium Greeneanum 10866]).

There are two individuals mounted on NDG 037326, both apparently sourced "from Petaluma, Miss Carlton, May 24, 1891" (on ticket in pocket). The left specimen is the more complete and is here designated as lectotype. This is the only known azalea specimen seen by Greene whose gathering predates the protologue and was also purportedly collected within the species' described distribution. Both left (lectotype) and right (non-type) specimens are considered here to be a local form of the typical variety.

Rehder consistently used the term "comb. nov." when shifting epithets or changing ranks. Although he clearly based his new variety on *R. sonomense* Greene, with a citation of the basionym and a partially accurate paraphrasing of Greene's description, after the intraspecific epithet Rehder added "var. nov." It is therefore interpreted here as a newly proposed variety with same type and epithet as *Rhododendron sonomense* Greene.

Two names have been misapplied to *R. occidentale* var. *occidentale*. The first is *Rhododendron calendulaceum* (Michx.) sensu Hook. & Arnott, Bot. Beechey Voy. 362. 1839, not sensu Torr., Fl. N. Middle United States 1:425. 1824. The other is *Azalea calendulacea* Michx., sensu G. Bentham, Plantae Hartwegianae 321, 1848, not sensu Michaux, Fl. Bor. Amer. 1:151. 1803. Both of these misapplications were based on specimens (!) collected near the coast by David Douglas (probably San Francisco Bay region) and T. Hartweg ("*in uliginosus prope* Santa Cruz"), respectively.

The typical variety, *Rhododendron occidentale* var. *occidentale*, occurs in the Outer North Coast Ranges, from northern Mendocino County south to northern Monterey and San Benito counties, California.

Rhododendron occidentale (Torr. & A. Gray) A. Gray var. **californicum** (Torr. & A. Gray in Durand) Hrusa, comb. et stat. nov. Basionym: *Azalea californica* Torr. & A. Gray in Durand, Plantae Prattennianae Californicae, J. Acad. Nat. Sci. Philadelphia ns 3(2):94, June 1855, not *Azalea californica* (Hook.) Kuntze, Rev. Gen. 2:387. 1891. —Type: USA. "Nevada, California, *Henry Pratten*" *s.n., s.d.* (lectotype: PH!, here designated).

An *Azalea* specimen at PH! with an annotation by Durand attributing the epithet "californica" to Gray and labeled as "*Azalea californica* T. & G.," is here designated as lectotype for *Azalea californica* Torr. & A. Gray in Durand (Durand 1856). The protologue indicates the specimen was collected in "shady hills along Deer Creek" without mention of collection date or further locality information. While the lectotype label has written on it only "Nevada, California" without the locality "Deer Creek," in the introduction to "Plantae Pratennianae" Durand equated these locations by describing Pratten's collecting localities as "in the vicinity of Nevada, a place situated along Deer Creek ..." According to Durand (1856), Pratten's Deer Creek collections were taken in 1851. This proposed lectotype of *Azalea californica* Torr. & A. Gray ex Durand (PH!) is matched by the SNPR azalea.

The variety occurs in the Interior North Coast Ranges, the Peninsular Ranges, and the Sierra Nevada of California.

Rhododendron occidentale (Torr. & A. Gray) A. Gray var. *paludosum* Jeps., Man. Fl. Pl. Calif., 741. 1925. —Type: USA, California, Humboldt Co., "Fortuna to Eureka, filling sedgy bogs in the meadows near Loleta", 1902, *Jepson #1916* (holotype: JEPS!).

The holotype of *Rhododendron occidentale* var. *paludosum* Jeps (JEPS!) is unequivocally representative of the KR azalea.

This variety occurs in the Klamath Ranges of Mendocino Co., California, to Douglas Co., Oregon.

Incertae sedis: *Azalea nudiflora* var. *ciliata* Kellogg, Proc. Calif. Acad. ser. 1, 1:60. 1855 (published 1873). (*n.v.*, probably destroyed 1906). Reported by Kellogg as "from the interior", the specimen is not currently extant.

Key To Varieties

1. Leaf abaxium generally with unicellular pubescence, not obscuring surface; multicellular trichome type on mid-vein or secondary veins of one kind, glandular or eglandular; corolla buds and open corolla white, often with a blush of pink at the lobe summits, rarely with a light pink blush in the tube, the tube otherwise white to greenish-white. Sierra Nevada, Peninsular Ranges, (inner North Coast Ranges as introgressants), California. . . . . var. *californicum*

1′ Leaf abaxium generally glabrous, rarely with a few unicellular hairs; multicellular trichomes on the midvein mixed eglandular and glandular types; corolla buds and open corolla tube and veins usually colored dark to light pink, or less often pure white, often with a pale to dark pink pigmentation in the limb

2. Abaxial secondary veins with multicellular trichomes, these often mixed eglandular and glandular types; tertiary veins often with associated multicellular trichomes; dormant bud bracts densely pubescent, the margin trichomes glomerate; twigs of the current season densely unicellular pubescent. Outer North Coast Ranges, California . . . . . var. *occidentale*

2′ Abaxial secondary veins generally lacking multicellular trichomes, or of a single type; absent on the tertiary veins; dormant bud bracts glabrous or thinly pubescent, the margins most often ciliate, occasionally glomerate; twigs of the current season glabrous to subglabrous. Klamath Ranges of California and Oregon, from Humboldt Co., California north to Winchester Bay, Oregon . . . . . var. *paludosum*

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Appendix 1

Georeferenced List of *Rhododendron occidentale* Populations

Population numbers correspond to those in Figure 1. Elevations are given in meters. All counties are in California except where specified. Representative vouchers are housed at CDA and DAV.

Pop.	County	Latitude/longitude	Elevation	Description
2	Napa	38°38′49″N, 122°21′42″W	175	Pope Creek, 3.5 km E of Pope Valley Rd–Chiles Valley Rd junction
4	Napa	38°32′45″N, 122°21′30″W	240	Chiles Valley Rd, 2 km N of junction of Hwy 128
4A	Napa	38°33′00″N, 122°22′00″W	245	Chiles Valley Rd, 2.5 km N of junction of Hwy 128
6	Napa/Lake	38°41′30″N, 122°26′47″W	200	Butts Cañon, between Pope Valley and Middletown
9	Napa	38°29′10″N, 122°23′18″W	80	Hwy 128 at Conn Dam (Lake Hennessey)
12	Josephine, OR	42°03′15″N, 123°44′45″W	510	Lone Mountain (Wimer) Rd, 3.5 km SW of O'Brien
15	Josephine, OR	42°14′00″N, 123°40′15″W	390	Eight Dollar Mountain Rd, ~7 km W of U.S. 199
25	Douglas, OR	42°55′10″N, 123°30′30″W	400	Cow Creek, ~1/2 km upstream of bridge on Hwy 321, 10 mi E of Riddle, OR
31	Del Norte	41°49′30″N, 123°59′30″W	500	French Hill Rd, ~4 km NE of U.S. 101
33	Sonoma	38°20′00″N, 122°26′00″W	280	Middle Nathanson Creek, 1.5 km above Gehricke Rd terminus
34	Colusa	39°17′30″N, 122°33′30″W	475	Cedar Mountain Rd, confluence Little Stony Creek and Frenzel Creek, Mendocino National Forest
36	Sonoma	38°34′21″N, 123°01′12″W	165	Gilliam Creek, canyon below Redwood Lake, Austin Creek State Recreation Area immediately N of Armstrong Redwood State Reserve
37	Marin	37°54′45″N, 122°36′13″W	380	Mount Tamalpais, Bootjack Creek approx. 1 km E of Bootjack Camp parking
42	Mendocino	39°10′15″N, 123°40′00″W	35	Navarro River, 7 km upstream Hwy 1
43	Trinity	40°24′00″N, 123°07′00″W	1100	State Hwy 36, ~9 km W of Wildwood, W fork Salt Creek, Telephone Ridge
45	Sonoma	38°20′30″N, 122°26′00″W	350	Hogback Mountain , headwaters of Agua Caliente Creek
46	Sonoma	38°35′00″N, 122°37′45″W	275	Franz Valley School Rd, immediately W Napa/Sonoma Co. line
49	Plumas	39°47′30″N, 121°27′15″W	500	Hwy 70, 1/8 mi W of Pulga Rd
50	Plumas	40°00′38″N, 120°59′33″W	1165	Plumas National Forest, Butterfly Valley Botanical Area, Darlingtonia bog
51	Trinity	41°07′15″N, 122°39′00″W	1530	Unnamed tributary, headwaters East Fork Trinity River, N side Bonanza King Ridge, ~9 km N of Bonanza King Lookout
52	Humboldt	39°54′50″N, 123°39′00″W	1000	Red Mountain,, headwaters of School Section Creek
53	Eldorado	38°54′30″N, 120°38′40″W	1250	UC Blodgett Experimental Forest, Mutton Creek
55	San Benito	36°42′40″N, 121°25′25″W	430	Gabilan Range, Pescadero Creek, Grass Valley, ~10 km S of Hollister
56	Santa Cruz	37°10′00″N, 122°13′00″W	340	Big Basin State Park,, Blooms Creek.
57	Trinity	41°06′16″N, 122°42′11″W	950	Scorpion Creek, approx. 3 km SE of Hwy 3 E of Trinity Campground
58	Humboldt	40°55′00″N, 124°04′15″W	75	McKinleyville, Azalea State Reserve
59	Humboldt	41°13′00″N, 124°06′00″W	155	Stagecoach Hill, Kane Rd approx 1 km E of U.S. 101
60	San Diego	33°20′00″N, 116°53′00″W	1500	Upper Doane Valley, Palomar Mountain State Park
61	San Diego	32°58′15″N, 116°36′00″W	1650	Azalea Glen, Cuyamaca Rancho State Park
62A	Riverside	33°47′45″N, 116°43′40″W	1800	San Jacinto Mountain,, Azalea Trails Girl Scout Camp, head of Dark Canyon.
62C	Riverside	33°47′45″N, 116°45′00″W	1600	San Jacinto Mountains , Fuller Mill Creek, along Hwy 243
64	Lake	39°10′00″N, 122°30′15″W	525	Intersection of Kilpepper Creek and Bartlett Springs Rd
65	Placer	39°03′30″N, 120°33′30″W	1600	Placer Redwood Grove
66	Tulare	36°45′00″N, 118°58′15″W	2000	Sequoia National Park, Grant Grove
68	Mariposa	37°44′00″N, 119°33′30″W	1300	Yosemite National Park, Happy Isles
69	Mariposa	37°30′45″N, 119°36′20″W	1400	Yosemite National Park, Mariposa Grove
71	Napa	38°33′40″N, 122°24′30″W	500	Las Posadas State Forest, upper Moores Creek